

Nitrogen Metabolism in the Soy Bean

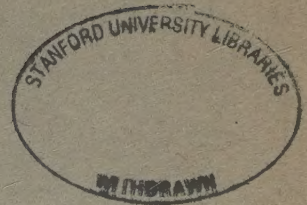
DISSERTATION

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE GRADUATE
SCHOOL OF THE OHIO STATE UNIVERSITY

BY

JAMES E. WEBSTER, B. S., in AGR.

The Ohio State University
1927



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UNCLASSED

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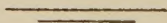
PREFACE

This paper is concerned with a study of the variation in percentages of various nitrogenous fractions of the Soy Bean (*Glycine Max*) when grown under different environmental and nutritional conditions. Analyses were made of the various organs at different stages of growth, and the data applied to a discussion of the nitrogenous changes accruing during the life cycle of the plant.

The writer wishes to express his indebtedness to Dr. T. G. Phillips for suggesting the problem and the helpful criticism he has given; to Dr. Sophia Eckerson, Dr. H. R. Kraybill and Dr. R. C. Burrell for their suggestions and criticisms; and to Dr. William Crocker for the aid and use of the equipment of the Boyce Thompson Institute for Plant Research where the analytical part of this problem was carried out.

TABLE OF CONTENTS

	Page
Introduction.....	5
Methods of Analyses.....	8
Experimental data.....	14
Summary.....	32
Conclusions.....	34
Literature Cited.....	35
Autobiography.....	38



NITROGEN METABOLISM IN THE SOY BEAN

INTRODUCTION

In attempting to solve some of the problems connected with nitrogen metabolism in plants, it was decided to determine the simpler nitrogenous constituents of some plant and to follow the changes in these compounds throughout the life cycle of the plant when grown under different conditions of nitrogen supply. Most of the simpler nitrogenous compounds of plants are water soluble (11). The work which this article describes, therefore, was confined mainly to the determination of the more important water soluble forms of nitrogen. For comparison, the percentages of total and insoluble nitrogen were also determined. The results thus secured have been used to throw light on the probable course of protein metabolism in green plants, and the functions of certain of the more abundant soluble forms of nitrogen.

The soy bean (*Glycine Max*) variety Peking (18) was selected for this work, since it is of medium height, is high in nitrogen and matures relatively early.

Emmerling (7) has reviewed the earlier literature of this subject very carefully and also gives an interesting discussion of his own work with *Vicia faba*. He analyzed plants on nine different dates making separate analyses of various parts such as the roots, stems, leaves, pods and seeds. He found that the seeds secured their nitrogen at the expense of the entire plant and that the leaves and hulls seemed to act as reserve storage organs for amids. A discussion of the literature on this subject up to a somewhat later date is given by Schulze (21).

Andre (1) has analyzed the leaves of Chataigner Common at different stages of growth and found that amids decreased up until blooming, then increased until the falling of the leaves. He raises the question, since there is so little nitrate present, as to what is the source of the nitrogen used in protein synthesis in this particular plant. Petrov (16) cultivated maize plants in sterile tubes under different conditions of nutrient supply and analyzed various parts of the plants for simple nitrogenous compounds. He concludes from his results that nitrates are reduced to ammonia during the oxidation of carbohydrates and that the ammonia so produced takes on the forms of various amid compounds, in some parts, of asparagine. Sure and Tottingham (27) concluded from a rather complete fractionation of the nitrogen of etiolated plants that alpha amino acids served for the production of amides in the nitrogen metabolism of etiolated plants.

Chibnall (5) has made quite extensive studies on nitrogen metabolism, especially of the leaf of the runner bean. He found that the leaf proteins changed but little after formation and that the fluctuations which occurred in the nitrogen compounds were mainly of the soluble forms. Some question has been raised by Nightingale (10) after his study of protein metabolism in various plants grown under different conditions particularly of light, as to the influence of the soluble forms of nitrogen on plant growth and he suggests that perhaps the ratio of insoluble nitrogen to carbohydrates is one of the most important in plants. Bambacioni (2) concluded from his analyses that all parts of the plants manufactured organic nitrogen compounds and that perhaps nitrogen compounds migrated from the roots to other parts of the plant. Thatcher (29) gives figures to show that in field grown soy beans the percentage of nitrogen in the roots decreased from the time of blossoming until maturity. From these figures he found that the nitrogen compounds of the roots may be drawn upon to supply other parts of the plant. Jodidi (9) working with etiolated seedlings of corn followed the decomposition of the proteins in

the seedlings by analyzing for various water soluble forms of nitrogen. He finds that there is a steady increase in amid and amino nitrogen and a decrease of peptide nitrogen. However, the increase in amid nitrogen is not proportionally greater than that of the amino nitrogen. Work (30) has conducted extensive experiments having to do with the effect of the nitrogen supply on the growth of tomato plants. However, he attempted no fractionation of the nitrogen and interpreted his results mainly in relation to the carbohydrate nitrogen ratio.

Several experimenters have worked on the storage of nitrates in plants. Schulze (20) was unable to confirm the presence of nitrates in etiolated seedlings as he had previously reported. Petrie (15) reported finding nitrates stored in several plants and discusses how ammonia and nitrates may function in plants. Woo (29) made analyses of *Amaranthus Retroflexus*, giving the results for total, nitrate and amino forms of nitrogen and found there was an inverse relation between nitrogen and carbohydrates. This problem was carried still further by Campbell (4), who concluded from his work that weeds do not, as a rule, store nitrates as had been suggested in the previous work. Stowd (24) analyzed the leaves, stems, roots and nodules of soy beans grown under field conditions. He concluded that there was an increase in the nitrate content of the roots arrested photosynthesis and that nitrates increased in the sap, but not proportionally, when the nitrate supply was increased.

METHODS OF ANALYSES

Sampling.

The procedure of sampling required about one and one half hours. In the case of series (a) and (d) the sampling was started at 8 A.M. With the remaining series, sampling was begun at 7 A.M. First, the leaves with their petioles were removed, weighed and ground, then after the cotyledons, when present, were removed, the stems were cut off at the surface of the soil or sand, weighed and ground and finally the roots were removed, washed free of adhering soil or sand particles, dried by careful pressing between filter paper, weighed, and ground.

Grinding and Extracting.

As soon as weighed, the parts sampled were ground through a Nixtamal Mill, once in the case of leaves, and one or more times in the case of stems and roots, depending upon the coarseness of the material. This reduced the leaves to a pulp and the stems and roots to a pulp containing shreds of fibrous material.

Before any water was added to the resultant mixture two separate samples were removed for moisture and total nitrogen determinations.

The residue was then stirred with cold distilled water and the watery mass transferred to a filter cloth of closely woven muslin and allowed to drain after which it was squeezed dry by hand, washed once and then placed in a beaker, covered with boiling distilled water and set on a boiling steam bath and allowed to remain there for two hours after which it was removed, cooled somewhat and the water extract decanted. The residue on the cloth was now washed thoroly with cold distilled water and squeezed dry. This operation was repeated several times so that the material retained by the cloth and fibrous material was reduced to a minimum. This water extract was

then made up to some convenient volume varying from 200 to 1000 cc. depending upon the amount of material extracted. See Chibnall (6).

This method was found to remove practically all of the water soluble forms of nitrogen. As a check on this, several of the residues were extracted in a soxhlet with 95% alcohol for sixteen hours. Tests of this extract showed that the water extraction had secured from 98.5% - 99% of the amid nitrogen and 98% - 99% of the nitrates. Similar results have been secured by Tottingham, Schulz, and Lepkovsky (28) who conclude from their work comparing various types of extraction, that their results indicate the desirability of extracting with water as a general rule in fractionating the nitrogenous constituents of plant cells. Osborne and Wakeman (13) state that the greater part of the cell contents of spinach leaves may be secured by grinding the tissue in a Nixtamal Mill and extracting with water.

Clarifying.

Before the various determinations could be made on this extract it was necessary to remove the colloidal material and to accomplish this 50 cc. portions of the well-shaken extract were heated to 65° - 70° C for the leaves and stems, and to 70° or even 75° C for the roots in case the lower temperature did not flocculate the colloidal material. These flocculated samples were then centrifuged until the supernatant liquid was clear -- usually 10 - 15 minutes -- after which they were decanted and the flocculum broken up with 10 cc. portions of water and centrifuged twice more. Two separate samples were coagulated and then the whole, including washings, was made up to 200 cc.

The extracts at this time, except for a few root samples, were clear; the leaf extract usually being of an amber color, the stems a pale greenish and the roots of a brownish white shade. These extracts,

after samples for ammonia and amid determinations had been removed, were preserved with toluene until the amino nitrogen determinations were made.

Moisture.

As previously stated, two samples for moisture determinations were removed at the time of grinding and placed in paired watch glasses. These, after being weighed, were placed in a vacuum over which was held constant at 70° C and through which a continuous small stream of air was drawn. The samples were allowed to remain in the oven for five days after which they were removed, cooled in a desiccator and weighed. The samples were then placed in beakers, covered with water and allowed to soak for a day. They were then transferred to a 300 cc. Kjeldahl flask to be used for total nitrogen determinations.

The duplicates, as a rule, checked to within 0.6% to 1.0 % of each other.

Total Nitrogen.

The moisture samples after being transferred to Kjeldahl flasks were placed on a steam bath and evaporated to dryness under vacuum (19). The samples were then analyzed according to the official method modified to include nitrates with the following variations (12). To make the salicylic, sulphuric acid mixture, 80 grams of salicylic acid were dissolved in a five pint bottle of C. P. nitrogen free sulphuric acid. Then 40 cc. of this mixture were added to each sample. The samples were allowed to stand at least an hour and then five grams of sodium thiosulfate added and the mixture heated over a small flame for five minutes, after which they were allowed to cool and eight grams of sodium sulphate added and the heating continued until the samples were colorless. The samples were diluted with 300 cc. of distilled water, a few drops of rosolic acid added and the solution made alkaline with 100 cc. of 50% NaOH, the ammonia distilled over

into N/10 acid, and the excess acid titrated with N/10 NaOH.

Soluble Nitrogen.

Duplicate determinations were run on 50 cc. portions of the original extract after it had been clarified (see clarifying). These determinations were made in the same manner as the total nitrogen samples above.

Insoluble Nitrogen.

This percentage is secured by difference (total - soluble) consequently, the result combines the errors of the two separate determinations.

Ammonia Nitrogen.

For this determination a modified Folin's method (8) was used. In this determination 10 cc. samples of the clarified solution, 15 cc. of 52% K_2CO_3 and 5 - 8 drops of capryl alcohol were added to the tubes on one side of the Van Slyke Cullen, Urea apparatus. To the tubes on the other side were added 10 cc. of N/50 acid, 15 cc. of water, 6 to 8 drops of capryl alcohol and 3 or 4 drops of methyl red indicator. The apparatus was then connected and aerated at such a rate as would draw over 120 liters of water in an hour. The excess acid was then titrated in the tubes using N/50 alkali.

The results were calculated after subtracting the titration figures from those secured by running blanks with distilled water in place of the samples.

With this method it was found possible to secure 98.77% to 99.36% of the theoretical amount present in reagent quality ammonium sulphate. To secure the figures shown in the tables four determinations were run on each sample and the results averaged to get the final figures. The amounts of ammonia are how-

ever, so small that only when a series of determinations are run can any reliance be placed in the results.

Amid Nitrogen.

For these determinations an acid hydrolysis method suggested by Dr. Phillips (17) was used. Ten cc. portions of the sample were transferred to tubes of the Van Slyke Cullen apparatus, several small pieces of pumice and 0.6 cc. of concentrated H_2SO_4 added and the sample boiled gently under a reflux for two and one-half hours. The samples were then cooled, nearly neutralized with 2.2 cc. of 20% NaOH, then cooled again, and capryl alcohol and 20 cc. of 52% K_2CO_3 added and the system aerated as in the ammonia determination. Four determinations were made on each sample and the results averaged. While these figures may be considered as representing amid nitrogen, there is some question as to whether or not other compounds do not liberate ammonia under these conditions (14) and the results must be considered accordingly.

The difference between the ammonia titration and the amid titration gives the acid equivalent to the ammonia secured by hydrolysis.

Due to the small amounts of material measured it would seem differences of 15% - 20% are necessary to be considered significant.

Using pure asparagine a recovery of 94.5% to 96.0% of that calculated was secured by this method.

Nitrate Nitrogen.

The method used was a modified Devarda's alloy method. The method suggested by Strowd (23) has been found to be unreliable under certain conditions (3) so that a modification suggested by Dr. Phillips was used.

The residue from the amid determination was washed into a 800 cc. Kjeldahl flask using 100-135 cc. of

water. This was then boiled nearly to dryness, allowed to cool and one and one-half grams of Devarda's alloy added and the whole diluted with 300 cc. of distilled water. The samples were then distilled into N/50 acid containing methyl red as indicator. The excess acid was titrated with N/50 alkali after the samples had been boiled to expel CO_2 and cooled. Blanks were run using 20 cc. of 52% K_2CO_3 in place of the nitrate samples.

Where the percentages are low a difference of at least 20% would be required to be significant since the titration figures were small in such cases.

Using reagent quality NaNO_3 and carrying through the entire procedure including the acid hydrolysis, a recovery of from 97.6% to 101.4% of the theoretical amount of nitrogen was secured.

Nitrate Nitrogen.

The amount present in any case is so small that no practical quantitative method could be found that could be used with the colored plant solutions that were secured. However, for a rough qualitative test the Griess-Ilosovay method (26) was used and the results indicate more the presence or absence of the nitrites than any definite amount.

Amino Nitrogen.

This determination was made on the cleared solution (see clarifying) using the Van Slyke apparatus as described in Matthews (11). As in Spoehr's work (22) no correction was made for the ammonia present.

Other Nitrogen.

This percentage was secured by adding together the amino, nitrate and ammonia results and subtracting this total from the percentage of soluble nitrogen. It is at once apparent that this percentage includes the errors of the other determinations and this

fact must be taken into consideration in interpreting the results.

EXPERIMENTAL DATA

Series (A)

High Nitrogen Plants Grown in Soil.

The plants were all grown in the greenhouse during the first eight months of 1925. The soil series (A) was started on the first of January and continued for the first sixteen weeks of the year. In order to conserve space the pots containing the plants to be harvested from the ninth to the sixteenth week inclusive were started first, then as they were removed, their places were filled with some other plants to complete the cycle of sixteen weeks. For example, when the plants ten weeks old were sampled they were replaced with plants which would be harvested at the end of six weeks. These plants were grown in rich humus soil contained in twelve-inch unglazed pots. This soil had been sterilized before using and at no time were there any nodules observed on the roots. The temperature for this series of plants was kept within $\pm 8^{\circ}$ of 78° F. and the humidity correspondingly varied around 50. These plants were watered with tap water.

Condition of Plants at Some of the Different Stages
in Their Life Cycle.

Age (In weeks)

1. Plants $1\frac{3}{4}$ - 3" high. Only one pair of leaves were showing.
2. Plants $2\frac{1}{2}$ - 5" high. Two pairs of leaves were showing on the majority of the plants. (Nitrates at the rate of approximately ten grams per eight gallons of water were added to the two and three week plots at the beginning of the second week.)
4. Plants 7 - 8" high. First blossoms were showing on a few plants. (A nitrate solution of the same strength as used in "two" was added to the fifth, sixth, and seventh week pots.)
6. Plants 8 - 10" high. Pods 1" long or over were present on most plants.
8. Plants 10" high and over. Pods well-formed. Cotyledons were present and the plants green.
10. No appreciable change occurred in height after this period. Many pods yellow, some dry. A few pods were still forming.
12. Leaves considerably mottled. Most of the pods were dry.
13. Considerable red spider injury. Tips of some leaves burned. The pods were mostly mature.
15. Tips of many of the leaves dead. All nearly dead leaves were discarded when sampling.
16. Seeds from many pods had fallen out. Tips of many leaves were dead and the remainder were badly mottled.

SERIES (A)

TABLE I (LEAVES)

Percentage of fresh weight

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	Other	Mois- ture
1	1.0221	.5752	.4469	.0746	.1247	.0771	trace	+	.2476	85.50
2	.7996	.5895	.2101	.0792	.0604	.0213	.0045	+	.0660	86.15
3	.7456	.5600	.1856	.0727	.0778	.0519	.0090	+	.0261	87.46
4*	.9629	.7373	.2256	.1345	.0539	.0243	.0021	+	.0351	83.48
5	.7988	.6074	.1914	.1076	.0589	.0261	.0031	+	.0218	81.87
6	.8679	.6733	.1945	.0712	.0514	.0198	.0027	+	.0592	82.57
7	.9719	.8100	.1619	.0378	.0524	.0157	.0033	+	.0684	81.68
8	.9773	.8133	.1638	.0580	.0663	.0145	trace	trace	.0395	80.55
9	.9085	.7721	.1364	.0391	.0475	.0107	trace	trace	.0498	79.15
10**	.7725	.6395	.1330	.0245	.0467	.0105	trace	none	.0618	78.56
11	.7981	.6353	.1628	.0297	.0818	.0186	.0029	none	.0484	77.14
12	.8910	.6381	.2029	.0131	.1112	.0255	.0062	none	.0724	75.23
13	.8679	.6788	.1891	.0276	.1033	.0173	.0087	trace	.0495	75.10
14***	1.2190	.9870	.2320	.0308	.0850	.0387	.0071	none	.0704	72.24
15	.9503	.7432	.2071	.0553	.0666	.0336	.0077	none	.0775	69.45
16	1.0527	.8289	.2238	.0458	.0926	.0340	.0072	none	.0779	66.19

* First signs of blossoming

** Pods had begun to mature

*** Pods completely mature, seeds falling out.

TABLE II (STEMS)

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Amonia	Nitrite	Other	Mois- ture
1	.5250	.1264	.3986	.1209	.1508	.1196	None	+	.1169	92.99
2	.3227	.0842	.2385	.1456	.0632	.0263	.0031	trace	.0266	90.71
3			.2099	.1400	.0323	.0240	.0040	trace	.0326	91.11
4	.3791	.1658	.2133	.1728	.0414	.0290	.0009	trace	.0018	87.62
5	.3386	.1375	.2109	.1882	.0459	.0229	.0015	trace	.0247	87.29
6	.3419	.1145	.2274	.1569	.0567	.0263	trace	trace	.0138	86.23
7	.3308	.0643	.2665	.1321	.0698	.0470	.0026	+	.0620	84.12
8	.3846	.1399	.2477	.0868	.1212	.0519	trace	trace	.0367	79.10
9	.4135	.1856	.2279	.0876	.0762	.0585	.0015	+	.0626	76.62
10	.3721	.1943	.1778	.0695	.0695	.0364	.0021	trace	.0367	75.06
11	.5383	.2584	.2799	.1005	.1266	.0739	.0079	trace	.0449	74.57
12	.6278	.2479	.3799	.0390	.1598	.0329	.0031	trace	.1780	74.40
13	.5960	.1564	.4396	.0562	.1378	.0763	.0049	trace	.2417	73.26
14	.6276			.0719	.1514	.1016	.0045	trace		72.50
15	.4934	.1102	.3832	.0678	.1465	.0868	.0167	trace	.1522	74.33
16	.6219	.2280	.3939	.0792	.1661	.1033	.0064	trace	.1412	77.23

TABLE III (ROOTS)

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid Ammonia	Nitrite	Other	Mois- ture
1	.2766	.0865	.1901	.0787	.0646	.0536	trace	.0377	94.28
2	.2685	.1436	.1249	.0821	.0547	.0114	trace	.0277	92.61
3	.2445	.1488	.0957	.0626	.0340	.0127	trace	.0019	94.05
4	.2342	.1445	.0897	.0729	.0163	.0127	trace	.0001	92.45
5	.2164	.0909	.1255	.1119	.0352	.0055	-	.0216	92.65
6	.2219	.1259	.0960	.0656	.0252	.0063	trace	.0007	92.00
7	.2064	.1006	.1058	.0612	.0224	.0064	-	.0199	90.23
8	.1723	.0998	.0725	.0556	.0128	.0086	trace	.0041	91.28
9	.2323	.1355	.0968	.0612	.0334	.0141	trace	.0009	89.42
10	.2327	.1148	.1179	.0674	.0379	.0101	trace	.0126	90.07
11	.2618	.1109	.1429	.0555	.0406	.0218	trace	.0437	89.94
12	.2706	.1348	.1358	.0465	.0564	.0187	-	.0201	90.27
13	.3604			.0664	.0525	.0203	trace		88.28
14	.3234	.1912	.1312	.0651	.0348	.0919	trace	.0271	87.44
15	.2652	.1352	.1300	.0738	.0695	.0142	none	.0147	87.61
16	.2579	.1640	.0959	.0697	.0267	.0196	none	.0032	88.55

SUMMARY OF SERIES (A)

The summary presented at the end of the data for each series was secured by a study of the tables here published and from graphs prepared from these data.

The variations in the percentages of total, insoluble and soluble nitrogen in the leaves, stems and roots are quite comparable with one another with the exception of the soluble percentage in the leaves. The curves for total and soluble nitrogen forms show two low points; one, two to three weeks after germination when the stored reserve nitrogen seems to be greatly reduced and before the plant has itself begun to elaborate many nitrogen compounds, and the other at the stage when the seeds are nearly full grown. It is interesting to note that the soluble nitrogen is generally higher in the stems than in any other part and that there is a notable increase there after the beans have begun to mature.

The percentages of nearly all forms fluctuate greatly at the sixteenth week when notable degeneration had begun.

The nitrates as a rule are much higher in the stems and roots than in the leaves. The relation between the various parts is in general similar, with the roots showing the greatest discrepancies. The various parts of the plant are lowest in nitrates at about the time of seed maturity (tenth to twelfth weeks) but due to the addition of nitrates to the earlier weeks too much importance should not be attached to the variation up to the seventh week.

Curves for the amino nitrogen run nearly parallel in the case of the leaves and stems, but that of the roots diverges widely from the other two. The stems show much the greatest range in percentage. Amino nitrogen is lowest around inception of visible flowering and highest at that period when the plants are nearly mature.

There is no striking comparison between the curves for amino and amid nitrogen though it is true that any great variation in the amino nitrogen is usually reflected in the amid percentage. The fact that the ratio of amid to amino nitrogen in the stems is much smaller than in either the leaves or roots is of interest. The ammonia figures being small have not any great significance altho they are lowest around the eight to tenth week and highest during the latter weeks when degeneration is greatest. Nitrites are present in the leaves during the earlier stages, but their appearance in other parts is questionable.

"Other" nitrogen figures are quite variable and at times show a negative value, possibly due to an incomplete recovery of nitrates in the soluble fraction. There is, however, a remarkable increase in this form in the stems after the tenth week, the peak being reached at the thirteenth week.

Series (B) and (C).

The high nitrogen series (B) and the low nitrogen series (C) plants were grown during the months of May, June and July. Due to the warm weather however, no attempt was made to keep a uniform temperature or humidity.

They were grown in clean, new, quartz sand contained in two-gallon glazed jars and were set in glazed saucers which were kept about one-half full of water. Before filling with sand, the pots were thoroly washed with tap water and then with distilled water. Only distilled water was used in watering plants grown in quartz sand.

The nutrient solution used was made up of the following C. P. chemicals dissolved in distilled water:

$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	2%
KNO_3	2%
$\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$	10%
K_2HPO_4	2%

For each pot of the high nitrogen plants, 25 cc. of each of the above solutions were mixed with 5 cc. of a 2% solution of iron citrate and the whole made up to 250 cc. and added weekly.

The solution used for the low nitrogen pots was identical with the high nitrogen one, with the exception that only 5 cc. of the 10% $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ was used.

Great difficulty was experienced in keeping green algae out of the saucers and usually after two or three weeks in the greenhouse, the saucers were covered with it.

High Nitrogen Series (B), Grown in Quartz Sand. Condition of Plants at Some of the Different Stages in Their Life Cycle.

Age (In weeks)

- 1 Plants 2" - 3" high. Only one pair of leaves were showing.
- 2 Plants 5" - 8" high. Plants dark green in color.
- 4 Plants 13" - 15" high. Leaves deep green and comparatively large.
- 6 Plants 23" - 25" high. Leaves dark green. A very few plants were just beginning to bloom.
8. Plants 28" - 38" high. Plants very green.
- 10 Plants same average height as the preceding stage. Leaves green, but showed considerable red spider injury. Beans about 2" long on most plants.

TABLE I (LEAVES) Percentage of Fresh Weight

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	Other	Mois- ture
1	.9781	.5127	.4654	.0815	.1344	.0464	.0185	++	.2310	81.59
2	.7821	.5510	.2310	.0901	.0759	.0147	.0091	++	.0559	87.44
3	.7080	.5301	.1779	.0773	.0610	.0101	.0058	++	.0338	87.88
4	.7342	.5833	.1509	.0299	.0598	.0119	.0054	++	.0558	85.07
5	1.0100	.8527	.1573	.0271	.0602	.0134	.0028	++	.0672	84.09
6*	.8950	.7045	.1905	.0526	.0745	.0140	.0027	++	.0607	83.16
8	.6771	.5554	.1217	.0189	.0498	.0093	.0054	+	.0476	82.02
10	1.0007	.7896	.2111	.0261	.1039	.0123	.0138	none	.0673	75.83

TABLE II (STEMS)

1	.5758	.1583	.4175	.0577	.1912	.1399	.0116	faint	.1630	92.75
2	.4142	.0921	.3221	.1365	.1696	.0579	.0064	faint	.0096	91.55
3	.2595	.0491	.2104	.1374	.0454	.0243	.0030	?	.0246	90.72
4	.1880	.0880	.1000	.0416	.0325	.0110	.0072	faint	.0187	89.11
5	.2367	.1441	.0946	.0408	.0468	.0118	.0025	++	.0045	88.30
6	.2341	.0982	.1385	.0764	.0387	.0171	.trace	++	.0234	85.62
8	.1765	.0788	.0997	.0212	.0530	.0092	.0043	+	.0215	81.99
10	.3013	.1599	.1414	.0270	.0680	.0327	.0100	none	.0364	75.03

* First blooms were beginning to appear.

TABLE III (ROOTS)

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	Other	Mois- ture
1	.2478	.0772	.1706	.0215	.0842	.0285	.0157	+	.0492	95.66
2	.2059	.0491	.1568	.0868	.0425	.0069	.0131	+	.0144	95.29
3	.1795	.0759	.1036	.0631	.0173	.0079	.0094	faint	.0148	94.54
4	.1614	.0821	.0793	.0340	.0212	.0045	.0019	faint	.0222	90.76
5	.1834	.1201	.0633	.0307	.0294	.0027	.0032	+	.0000	91.06
6	.2069	.1252	.0817	.0456	.0241	.0066	.0027	+	.0093	90.46
8	.1526	.1137	.0389	.0203	.0177	.0036	.0015	+	.0006	89.03
10	.1758	.1044	.0714	.0270	.0247	.0071	.0099	faint	.0098	88.86

SUMMARY OF (SERIES B)

In these plants the curves for the total nitrogen in leaves, stems and roots are quite similar and reach their lowest point at time of flowering. The soluble nitrogen curves are quite similar, but reach their extreme variation at a slightly different time from the total nitrogen curves. The insoluble nitrogen curves for the leaves and stems are similar to the corresponding total nitrogen curves, but the curve for the roots is quite variable.

The nitrate nitrogen curves are quite similar for the various parts and are lowest just after blossoming time. All parts have quite high percentages of nitrates the first three weeks after which a marked decline takes place.

The amino nitrogen curves are rather irregular altho they somewhat resemble the nitrate curves. As with the nitrates there is a marked decline at the third week. The amid nitrogen in the leaves and stems is somewhat proportional to the amino nitrogen while in the roots the figures are too small to have any particular significance.

The amount of ammonia varies greatly and perhaps the only significant fact from its curves is that it is lowest around time of flowering.

"Other" nitrogen fluctuates greatly in the various parts and with little apparent correlation with any other form.

Low Nitrogen Series (C), Grown in Quartz Sand. Condition of Plants at Some of the Different Stages in Their Life Cycle: (Age in weeks)

1. Plants 2"-4" high. Only one pair of leaves.
3. Plants 6"-8" high. Leaves green and mostly small.
5. Plants 10" - 14" high.
6. Plants 15"-18" high. Plants very light green. Leaves were only about one-half the size of those of the high nitrogen plants.
8. Plants 15" - 25" high. First blossoms were appearing. Leaves were light green.
10. Plants about 30" high. A few plants had runners. Leaves small and light green. Some pods 1" - 2" long. Some red spider injury to the leaves.

TABLE I (LEAVES) Percentage of fresh weight

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	Other	Mois- ture
1	1.2006	.8000	.4006	.0364	.1260	.0436	.0164	+	.2218	82.82
2	.7013	.5189	.1824	.0478	.0559	.0142	.0099	+	.0688	87.42
3	.6112	.4632	.1480	.0506	.0337	.0073	.0063	+	.0574	88.18
4	.5612	.4501	.1101	.0242	.0646	.0099	.0056	+	.0157	85.66
5	.6353	.4977	.1376	.0067	.0704	.0094	.0022	+	.0583	83.76
6	.4508	.3817	.0691	.0152	.0471	.0063	.0057	none	.0011	79.45
8*	.5146	.4382	.0764	.0104	.0319	.0068	.0026	none	.0315	81.31
10	.4703	.3478	.1226	.0176	.0614	.0179	.0081	none	.0355	77.61

TABLE II (STEMS)

1	.5680	.1713	.3967	.0217	.1913	.1359	.0067	+	.1770	92.88
2	.2406	.0448	.2379	.0916	.0612	.0533	.0071	+	.0780	91.63
3	.1577	.0646	.1958	.1119	.0379	.0200	.0100	+	.0360	90.40
4	.1660	.0768	.0931	.0386	.0473	.0073	.0044	+	.0028	82.29
5	.1507	.1100	.0892	.0164	.0574	.0103	.0018	none	.0136	84.58
6	.1377	.0734	.0407	.0079	.0458	.0055	.0012	none	.0142	76.80
8	.1748	.1015	.0643	.0192	.0398	.0073	.0079	none	+.0450	88.08
10			.0733	.0347	.0350	.0076	.0106	none	-.0068	76.61

* First blossoms were appearing.

TABLE III (ROOTS)

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	other	Mois- ture
1	.2373	.0920	.1453	.0052	.0707	.0332	.0128	+	.0566	95.67
2	.1188	.0500	.0688	.0279	.0274	.0068	.0056	+	.0079	95.54
3	.1877	.1107	.0770	.0351	.0172	.0035	.0063	faint	.0184	95.76
4	.1388	.0854	.0534	.0224	.0230	.0027	.0036	faint	.0044	92.45
5	.1442	.0949	.0493	.0105	.0298	.0066	trace	faint	.0090	92.08
6	.1457	.0896	.0561	.0060	.0145	.0012	.0023	faint	.0333	90.60
8	.1330	.0688	.0642	.0169	.0189	.0028	.0031	none	.0252	80.16
10	.1315	.0799	.0516	.0196	.0231	.0041	.0053	none	.0036	89.98

SUMMARY OF SERIES (C)

The curves for total and insoluble nitrogen follow each other very closely in each of the different parts, although the relation between the various parts is not so marked. After the first three weeks there are only small variations in these fractions in the stems and roots.

The curves for the soluble fraction follows a little less closely the total nitrogen curves and is lowest just before inception of flowering.

The nitrate curves with the exception of one point in the leaf curve, vary similarly and are noticeably lowest just before the appearance of flowers; after which there is a considerable increase especially in the stems.

The amino nitrogen curves only follow one another in general. In this particular series the fluctuation in the amino and nitrate nitrogen curves seem to run inversely and especially is this true in the stems.

The amid nitrogen is very low often being less than the ammonia nitrogen. In all parts the percentage of amid nitrogen constitutes only a very small fraction of the amino nitrogen percentage.

Nitrites are found at first in the various parts but later only the leaves have any and even here they disappear about the time flowering begins.

The "other" nitrogen curves varies greatly and do not seem to follow any particular form.

A COMPARISON OF SERIES (B AND C)

While a difference in the level of nitrogen supply does markedly influence the physiological appearance of the plants, in this case at least, it does not seem to have greatly affected the growth habits.

The main chemical differences are shown in the considerably lower total nitrogen content of the low nitrogen plants and the presence of nitrites in

the stems and roots at only a few periods. One other striking feature of the low nitrogen plants is their very much lower ratio of amid to amino nitrogen as compared to the same ratio in the high nitrogen plants. This statement is true for the leaves, stems, and roots. The percentage of moisture is also slightly higher in the low nitrogen plants.

SERIES (D)

Low Nitrogen Plants Grown in Quartz Sand.

Nutrient Solution Minus Nitrates.

Series (D) was grown during July and August. This was a minus nitrates series and was grown in quartz sand contained in glazed jars both of which had been sterilized in a steam sterilizer before the beans were planted. The nutrient solution used was made up from reagent quality chemicals and consisted of 25cc. of each of the following solutions and 5 cc. of the iron citrate solution the whole being made up to 250 cc. and applied weekly.

$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	2%
KCl	2%
$\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$	4%
$\text{K}_2\text{H PO}_4$	2%

Condition of Plants at Some of the Different Stages in Their Life Cycle

Age (In weeks)

- 2 Plants around 4" high. Plants were dark green.
- 6 Plants 7" - 9" high. Leaves very yellow. Some red spider injury to leaves.

TABLE I (LEAVES)
Percentage of fresh weight

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	Other	Mois- ture
2	1.1189	.7774	.3415	none	.0885	.0470	.0069	trace	.2461	85.22
4	.8426	.6587	.1839	none	.0637	.0248	.0045	none	.1157	84.33
6	.3881	.2783	.1098	none	.0270	.0146	.0056	none	.0772	79.01

TABLE II (STEMS)

2	.6286	.1617	.4669	none	.2492	.1681	.0872	trace	.1305	92.18
4	.3923	.1333	.2590	none	.1193	.0330	.0038	none	.1359	88.66
6	.1692	.0699	.0993	none	.0194	.0115	trace	none	.0799	77.05

TABLE III (ROOTS)

2	.2134	.0952	.1182	none	.0600	.0319	.0086	trace	.0496	95.26
4	.1582	.0983	.0599	none	.0343	.0128	.0068	none	.0188	94.17
6	.0961	.0464	.0497	none	.0097	.0044	.0017	none	.0383	92.68

SUMMARY OF SERIES (D)

The analyses of this series show but one significant fact; namely, that there is a constant decrease in nitrogen in all parts of the plant and that this decrease is shared alike by all of the forms for which analyses were made. The only exception to this statement is the "other" nitrogen of the roots which showed an increase at the sixth week.

SERIES (E)

Low Nitrogen Plants Grown in Quartz Sand
No Nutrient Solution Used.

The plants of series (E) were grown during July and August in quartz sand prepared in the same manner as that in (B) and (C). These however, were not given any nutrient solution and consequently were dependent upon the material stored in their cotyledons for growth.

Condition of the Plants at Some of the Different Stages in Their Life Cycle.

Age (In weeks)

1. Plants 3" - 4" high. Leaves very green.
2. Plants around 4" high. Leaves green but tips slightly heat burned.
4. Plants around 6" high. Leaves green.
6. Plants around 6" high. Many leaves yellow, some mottled.

TABLE I (LEAVES)
Percentage of fresh weight

Wks.	Total	Insol.	Solu- ble	Nitrate	Amino	Amid	Ammonia	Nitrite	Other	Moisture
1	1.1776	.7616	.4160	none	.1072	.0640	.0076	+	.3012	86.62
2	1.1831	.7820	.4011	none	.1290	.0791	.0137	none	.2584	81.49
4	1.1075	.8835	.2240	none	.0980	.0399	.none	none	.1260	79.38
6	.9692	.7489	.2203	none	.1073	.0342	.0043	none	.1087	73.57

TABLE II (STEMS)

1	.6308	.1653		none	.2358	.1724	.0050	trace	.2247	92.57
2	.7389	.1681	.5708	none	.2907	.1978	.0190	none	.2611	91.09
4	.4058	.1457	.2601	none	.0873	.0737	.none	none	.1728	85.06
6	.3313	.1901	.1412	none	.1033	.0188	.0039	none	.0340	78.69

TABLE III (ROOTS)

1			.1468	none	.0631	.0402	.0096	trace	.0741	
2	.2091	.0239	.1852	none	.0746	.0530	.0107	none	.0999	95.91
4	.1888	.1095	.0793	none	.0295	.0154	.0067	none	.0431	93.58
6	.1678	.1127	.0551	none	.0355	.0265	.0066	none	.0130	92.98

SUMMARY OF SERIES (E)

The total and soluble nitrogen curves for the leaves, stems and roots show very much the same fluctuations, there being, after the second week, a continual fall. The insoluble nitrogen, however, is quite variable. Points of particular interest are the increase in nearly every form of nitrogen at the second week and the small change in the insoluble fraction at all times in the leaves and stems.

The various soluble forms with the exception of the amino nitrogen at the sixth week show somewhat of a drop after the second week.

The trace of nitrites present in the various parts at the end of the first week is interesting although no explanation of their presence can be given.

SUMMARY

A point of particular interest is brought out by the weekly variations in the percentages of total nitrogen as shown in series (A), (B) and (C). Thus it may be seen that there is, as a rule, a weekly variation in no particular direction and that this would not be shown by data representing only one or two samplings during the life cycle of the plant. It would seem that this point has been overlooked by many in interpreting chemical analyses.

The fact that the supply of nitrogen may be varied over such wide limits as shown in (B) and (C) and yet not materially disarrange the growth cycle is of interest in discussing the carbohydrate-nitrogen ratio. Data concerning the carbohydrate fluctuations would, however, be necessary before this could be discussed fully.

From a consideration of the first series (A) and some data unpublished it would seem that the addition of nitrates results in a temporary increase in nitrate storage in all parts of the plant. This is soon, however, reduced to normal and this without any noticeable increase in the other soluble forms.

It is worth noting that the soluble nitrogen is relatively much more abundant in the stems and roots than in the leaves of all the plants for which data is presented.

There is certainly no evidence to show that under these conditions, there is any tendency for any of the forms of nitrogen here considered to group themselves in a particular part of the plant with a resultant loss to any other part. From these data there also appears no reason for assuming that all parts of the plant may synthesize organic nitrogen compounds. It must be borne in mind that in considering these data the plants were grown under somewhat abnormal conditions and this may account for some discrepancies between this work and others, as for instance Thatcher's (27).

After carefully considering the results of the various series it seems impossible to consider that the amid forms of nitrogen are of any more importance in nitrogen metabolism than are the other amino acids. There does however, seem to be a tendency for the ratio of amid to amino nitrogen to increase when a large supply of nitrogen is available. This might be interpreted as showing that amid forms are of importance as soluble storage forms.

From a consideration of the tables given it would seem that the formation of nitrites represents an intermediate stage in nitrogen metabolism when nitrates are supplied to plants. On the other hand nitrites do not seem to be an intermediate stage in such changes when organic forms of nitrogen are used. Plants living on their stored reserves would come under this latter class.

Although the percentage of ammonia is very small in nearly every case there seems to be reason for concluding that ammonia is one of the intermediate stages in both the synthesis and destruction of proteins in plants.

The column marked "other nitrogen" represents a nearly unknown fraction of soluble nitrogen. It is hoped, when time is available to carry on work leading to a fractionation of this portion.

CONCLUSIONS

1. In the majority of cases studied the variations of the various types of nitrogen compounds in the roots, leaves and stems of any particular series, are quite similar.

2. From the figures here presented there is little or no evidence in support of the idea that the leaves, stems or, if present, the seeds, draw upon the reserve of the roots.

3. There is no particular evidence to show that amino acids having amid groupings are of any more importance in protein metabolism in plants than other amino acids, although there is some evidence to show that amid nitrogen may be one of the most important soluble storage forms of nitrogen.

4. Additional proof has been found for the idea put forward by several workers that ammonia is the first and last product of nitrogen metabolism in plants.

5. The oftener the plants are sampled the more irregular are the curves. This shows considerable daily fluctuation in the various forms of nitrogen in no uniform direction.

6. The results here presented tend to show that within a wide range, when the nitrogen supply is the only variable, only the general appearance and not the physiological cycle of the soybean is changed.

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AUTOBIOGRAPHY

I, James E. Webster, was born in Quaker City, Ohio, January 9, 1903. My elementary education was secured in the Quaker City and Norwich, Ohio public schools; and my secondary school education in the Norwich High School and Muskingum Academy. I secured the degree of B. S. in Agr. from Ohio State University in 1924. I was employed at the Boyce Thompson Institute, Yonkers, N. Y. during 1924-25. From there I returned to Ohio State University in 1926 as assistant in the Department of Agriculture Chemistry which position I held until the fall of 1927 when I accepted the position of Assistant Professor in Agricultural Chemical Research at Oklahoma A. and M. College. I returned to Ohio State University in the winter of 1928 and received the Degree of Doctor of Philosophy at the close of the winter quarter.

